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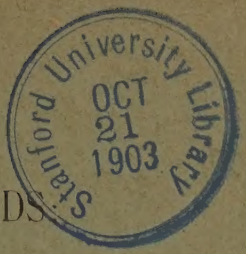

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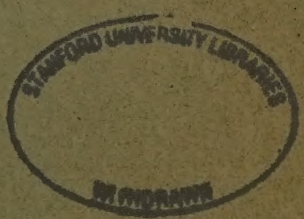
STUDIES IN PLANT HYBRIDS

THE SPERMATOGENESIS OF HYBRID COTTON

BY WILLIAM AUSTIN CANNON

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF PURE SCIENCE OF COLUMBIA UNIVERSITY



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Studies in Plant Hybrids: The Spermatogenesis of Hybrid Cotton

By William Austin Cannon

(With Plates 7 and 8)

Introduction

Perhaps the first suggestion that we have as to the possibility of crossing two or more less distantly related plants was given by Camerarius * in his letter to Valentini on the sexuality of plants. This was in the year 1694. In this letter Camerarius speculates as to the effect of crossing the hemp (*Cannabis*) and the hop (*Humulus*), wondering whether the posterity of the form used as the mother would by this cause be altered. He did not make the cross, and in fact it was not until 1760 that the first plant hybrid which was of use to science was made. In that year Kölreuter,† perhaps the greatest as he was the first of hybridizers, crossed *Nicotiana paniculata* ♂ and *N. rustica* ♀ and obtained fertile offspring, and thus began successfully the experimentation which he carried on with so great results for several decades.

The first plant hybrid thus empirically formed served a good purpose by providing incontestable evidence upon certain of the leading biological questions of the day. It fortunately was of the intermediate type, and thus the fact that both parents contributed equally to the formation of the hybrid offspring demonstrated at once the falsity of the evolution or preformation hypothesis, gave

* Camerarius, Ueber das Geschlecht der Pflanzen, translated from the Latin "De sexu plantarum epistola." (Ostwald's Klassiker der exakten Wissenschaften, 105 : 49. 1899.)

† Kölreuter, Vorläufige Nachricht von einigen das Geschlecht der Pflanzen. (Ostwald's Klassiker der exakten Wissenschaften, 41 : 30. 1893.)

[The preceding number of the Bulletin, Vol. 30, No. 2, for Febuary, 1903 (30: 75–132), was issued 28 F 1903.]

a strong support to the idea of sex in plants, and also pointed the way to the true conception of fertilization.

In addition Kölreuter, in the course of his long career as a hybridizer, arrived at other results that were of almost equal importance. For instance, he first pointed out the meaning of the visits of insects to flowers, and thus formed the basis for Sprengel's subsequent work, and he first showed the significance of nectar in this connection.* Kölreuter also introduced the "modern" methods in experimental research, as a single illustration taken from many will suffice to show. He had the mistaken idea that the part of the pollen necessary to insure fertilization was the fluid which he had observed about it and which he supposed originated in the interior of the grain and oozed out through the pores. He therefore was interested to learn how many grains were required to effect fecundation. To ascertain this he counted the grains in a flower and artificially pollinated with a varying but a known number. In *Hibiscus Trionum* he counted 4,863 grains, and found that only 50–60 were necessary to fructify more than 30 ovules. In *Mirabilis Jalapa* and *M. longiflora* two or three, or only one were required to fertilize the single ovule.

Kölreuter recognized all of the important characteristics of plant hybrids.† He learned that species-hybrids were as a rule intermediate and uniform, whichever race provided the male or female parent. He observed the fruitfulness of variety-hybrids, the sterility or lessened fruitfulness of those between species, the stronger growth of many hybrids and other phenomena. In his experiments Kölreuter used mainly the following genera: *Aquilegia, Matthiola, Dianthus, Melandrium, Linum, Malva, Lavatera, Lobelia, Nicotiana, Datura, Lycium, Verbascum, Digitalis* and *Mirabilis.*

About the time that Kölreuter was engaged in the experimental study which gave such remarkable results, Linné, ignoring that scientist's work, put forward theories in which hybrids played a prominent rôle. And this was done with hardly any experimental basis for his ideas. I refer to Linné's hypotheses regarding the origin of genera and species. According to Sachs (*l. c.*

* Sachs, Geschichte der Botanik, 440. 1875.

† Focke, Die Pflanzen-Mischlinge, 431. 1881.

114) the doctrine is in brief this, in the first place classes were created, by the subsequent mixing of the individuals of the different classes genera arose, and finally by the crossing of these genera species were differentiated.

It was not long however before some practical tests were made of the relation of the constancy of species to the possibility of deriving fertile hybrids by crossing them. Apparently without an adequate reason for his belief, Sprengel thought that crosses between distinct natural species might be fertile, and he accordingly is pleased to discover means by which plants may resist hybridizing, as for instance the difference in season of the maturation of the reproductive organs. This is considered by him as a miraculous adaptation for the preservation of the purity of the species.

The relation of Kölreuter's results and methods to the science of his time is so well given by Sachs that I shall quote a paragraph direct (*l. c.* 446): "Der allgemein theoretische Werth von Koelreuter's künstlichen Pflanzenbastarden ist gar nicht hoch genug anzuschlagen; die Vermischung der Eigenschaften der väterlichen und mütterlichen Form war der stärkste Beweis gegen die Evolutionstheorie und liess gleichzeitig einen tiefen Blick in das wahre Wesen der sexuellen Vereinigung thun. Auch ging aus Koelreuter's zahlreichen Untersuchungen sofort hervor, dass nur ganz nahe verwandte Pflanzen und auch diese nicht immer einer geschlechtlichen Vereinigung fähig sind, wodurch die vagen Vorstellungen Linné's für jeden Urtheilsfähigen sofort beseitigt wurden, wenn es auch immerhin noch lange dauerte, bis die Wissenschaft alle Vortheile aus Koelreuter's Untersuchungen zog. Die Pflanzensammler aus der Linné'schen Schule ebenso, wie die eigentlichen Systematiker am Ende des vorigen Jahrhunderts, hatten kein Verständniss für derartige Leistungen, ja Koelreuter's Ergebnissen zum Trotz, verbreiteten sich in der botanischen Literatur später unrichtige Vorstellungen über Bastarde und ihre Fähigkeit sich fortzupflanzen; den Gläubigen der Constanzlehre konnten die Bastarde ohnehin nur unbequem sein, sie störten ihnen die Reinlichkeit des Systems und passten zudem nicht recht zu der Annahme, dass jede Species eine 'Idee' repräsentire."

Whether hybrids between parents of different species are always sterile, and those between varieties always fertile, soon

became a question of great importance, since by this means it was hoped that a distinction between varieties and species might be had. Early in the nineteenth century the question was taken up by English and later by French botanists and since the facts were not well known the bearing of them on the question under discussion was also much in dispute. In England, Herbert * and Knight † discussed the relative fertility of species- and variety-hybrids, the latter author holding that hybrids from parents of different species are always sterile, but on the other hand that the varietal hybrids are always fertile. From the results of his experiments and study Knight concluded also that fruitful hybrids indicate that the parents are not distinct species, but varieties of the same species. Herbert on the contrary found that hybrids between different species are not seldom fertile. As to the significance of this he does not appear to think the parents were of the same species, but rather (*l. c.* 16) that they "have branched out from one common stock since the creation of the world." He further says: "If it be admitted, that diversity of species could have been produced by variations of soil, temperature, or humidity, it will be readily understood that such diversity might have been further multiplied by hybrid intermixture, as the species were brought together by the natural progress of their diffusion."

It will not be necessary for the purposes of this paper to enter more into the details of this phase of the "constancy of species" doctrine. The ground that Knight and Herbert threshed over in England was gone over again later (1862) in France by Naudin and Godron.‡ Finally, Darwin § has pointed out that not only is the fertility or sterility of a hybrid no criterion of the relationship of the parents, but that the hybrid may even be more fruitful than either pure parent.

Focke (*l. c.* 443) says that up to the time of Nägeli (1865) the knowledge of hybrids was unconnected and fragmentary, and that this author united and made coherent the results of the earlier hybridizers. Especially he drew consistent conclusions from the

* Herbert, On the Production of Hybrid Vegetables. Trans. Hort. Soc. Lond. 4: 15. 1820.

† Knight, Observations on Hybrids. Trans. Hort. Soc. Lond. 4: 367. 1821.

‡ Focke, Die Pflanzen-Mischlinge, 441. 1881.

§ Darwin, On the Origin of Species, Am. ed., 235. 1883.

facts gathered together by Gärtner. The general conception of hybrids held at the present is based on Nägeli's writings. He noted the tendency of hybrids to vary, and to revert, and he showed the value of the reciprocal cross, and, finally, he formulated the laws governing the action of hydrids. He also observed the behavior of hybrids in the second and later generations when they were inbred, although the significance of this was of course not appreciated by him.

At the time when the work of Nägeli was being published, that of Gregor Mendel * had been recently presented to the society at Brünn. His main theme was to account for the behavior of hybrids in the second and later generations. It will not be necessary in this place to present a full account of Mendel's laws because since their rediscovery by de Vries,† Correns,‡ and Tschermak § they have been put out unabridged both in their original form ‖ and in English translation,¶ and have been explained by several writers.** For convenience of reference I shall however give a brief outline of Mendel's results and conclusions taken mainly from my previous paper.††

When one pure form (*A*) is crossed with another pure form (*a*) the hybrid of the primary cross shows the *A* characters only. When, however, the hybrid plants of this generation are fertilized

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* Mendel, Versuche über Pflanzen-Hybriden. Abh. Naturf. Ver. Brünn, 4: 3. 1866.

† De Vries, Das Spaltungsgesetz der Bastarde. Ber. Deuts. Bot. Gesells. 18: 83. 1900; Ueber erbungleiche Kreuzungen. Ber. Deuts. Bot. Gesells. 18: 435. 1900; Sur les unités des caractères spécifiques et leur application a l'étude des hybrides. Rev. Gèn. Bot. 12: 257. 1900.

‡ Correns, G. Mendel's Regel über das Verhalten der Nachkommenschaft der Rassenbastarde. Ber. Deuts. Bot. Ges. 18: 158. 1900; Gregor Mendel's "Versuche über Pflanzen-Hybriden" und die Bestätigung ihrer Ergebnisse durch die neuesten Untersuchungen. Bot. Zeit. 58^2: 229. 1900.

§ Tschermak, Ueber künstliche Kreuzung bei *Pisum sativum*. Zeitschr. Landw. Versuchswesen Oesterr. 5: 465. 1900; Ber. Deuts. Bot. Gesells. 18: 232. 1900.

‖ Ostwald's Klassiker der exakten Wissenschaften, No. 121. 1901.

¶ Jour. Roy. Hort. Soc. Lond. 26: 1. Au 1901.

** Bateson & Saunders, Experimental Studies in the Physiology of Heredity. Rep. Evolution Committee Roy. Soc., Report 1. 1902; Emerson, Agric. Exp. Sta. Neb. Rep. 15: 30. 1902; Spillman, Science, II. 16: 709. 1902; Pop. Sci. Month. 62: 269. Ja 1903.

†† Cannon, A Cytological Basis for the Mendelian Laws. Bull. Torrey Club, 29: 657. D 1902

among themselves and produce offspring the *a* characters are seen, and in a definite proportion to the form bearing the *A* characters. These constitute the hybrids of the second generation. If now the hybrids of the second generation are fertilized in such a manner that plants with *a* characters are crossed with those bearing the same characters, and likewise plants bearing *A* characters with forms like themselves, the resulting hybrids will behave in a manner characteristic of the respective cross. That is (1) the plants with *a* characters will be found to transmit those characters only, *i. e.* they are "fixed"; and (2) when the plants with *A* characters are fertilized with other plants bearing the same characters, that is to say, if inbred, two sorts of hybrids will result: one portion will bear only the *A* characters, which may be demonstrated by breeding as before, and one portion, apparently also with *A* characters only, will be found to vary just as the hybrids of the primary cross varied, *i. e.* this will be really mixed or hybrid. This general scheme may be better understood if tabulated as follows:

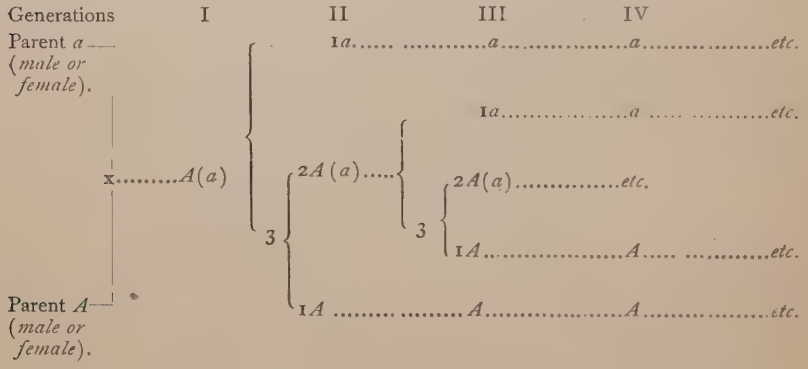

FIG. 1. Explanation: *A*, dominant character; *a*, recessive character; *A*(*a*), a hybrid having both characters of which (*a*) is masked by *A*.

Not only do the hybrids vary thus in a regular manner, but there is also a definite proportion of recessives (*a*), and dominants (*A*), as the table indicates. That is, referring to the table, in the second generation one fourth of the offspring is recessive (*a*), and three fourths apparently dominant (*A*), but really composed of two sorts (*A*(*a*)) one third of these being dominant (*A*), two thirds mixed (*A* (*a*)). The latter continue in the succeeding generation to vary just as the hybrids of the primary cross varied, one fourth of

their offspring being recessive, one fourth dominant and one half mixed.

The regularity in the variation as just described in the second and later generations is accounted for by supposing that the hybrids of the first generation organize germ-cells which are of pure descent, and that these unite in fertilization according to the laws of chance. Taking a specific case by way of illustration, we can imagine the following to take place, when the sex-cells of say the second generation hybrids ($A(a)$) meet each other in fecundation. The pollen, which is of pure descent, unites with the egg, which also is of pure descent, and the chances of union may be thus expressed: *A A; A a; a A; a a.* So that it happens, since the anther forms two sorts of germ-cells and the ovaries also two sorts, that in this way one half of the hybrids of say the third generation will be of mixed descent, and one half pure, the latter being equally recessive and dominant. The results, as calculated by the laws of chance, are thus seen to be precisely the same as what is found empirically to occur.

Such may perhaps be considered the more essential facts and conclusions of the discovery of Mendel, and upon them are based the two so-called "laws" of Mendel, namely, the law of dominance and that of the splitting of the hybrid race.

Mendel gives also, and in much detail, the results when the pure parents of hybrids are separated by more than one character, as, for instance, by *two* or *three*. But his experiments are too extensive to present here and I can not do better than to quote from the account of them by Bateson (*l. c.* 9). "In both sets of experiments the numbers of individuals and their constitution * * * were consistent with the hypothesis arrived at in the case of varieties differing in respect of one character, namely, that each male and female cell of the cross-bred is pure in respect of one character of each pair of characters, and is capable of transmitting this character to the exclusion of the opposite character; that the reproductive cells are, in the cross-breds, of as many kinds as there are possible combinations of pure characters (taken two or three together, as the case may be); and, finally, that each kind is represented in the cross-breds on the average in equal numbers."

In considering the probable bearing of Mendel's discovery on the biological sciences I can do no better than to quote at some length from Bateson's recent report, but of course I shall not attempt to present a summary of all of this author's conclusions.

Bateson says (*l. c.* 125): "With the discovery of the Mendelian principle the problem of evolution passes into a new phase. It is scarcely possible to overrate the importance of this discovery. Every conception of biology which involves a knowledge of the physiology of reproduction must feel the influence of the new facts, and, in their light, previous ideas of heredity and variation, the nature of specific differences, and all that depends on those ideas must be reconsidered, and in a great measure modified."

So much for the scope of the Mendelian principle. Bateson then cites specific examples, well known to breeders and scientists, which have hitherto been puzzling enough but which are apparently well explained by the discoveries of Mendel. Among these may be mentioned (1) the skipping of a generation, (2) the appearance in a pure strain of "rogues," and (3) the inability of breeders to fix by selection certain forms. The first is an integral part of the Mendelian conception and therefore need not be spoken of further here. The "rogues" which appear in apparently pure strains are supposed to be recessives, while in such instances the characters desirable for fixation are the dominant ones. And the last case will be best explained by the author himself. At page 131 he says: "It has long been known to breeders that certain forms cannot be fixed by selection indefinitely continued. In other words, though they produce offspring like themselves, they have also a large number which do not resemble them.

"A case of this kind is seen in breeding crested canaries. The kind of crest desired for exhibition can, according to canary-fanciers, be produced most easily by mating crested birds with non-crested, or plain heads as they are called. If it is supposed that the crested character is usually dominant, we have a simple explanation. When crested birds are bred together a number of birds are produced whose crests are coarse and stand up and others without crests. The latter are the recessives; the former we may suppose to be the pure dominants. What the fancier wants is a crest composed of long feathers lying evenly down over the head.

These may be the heterozygotes,* and consequently cannot breed true or be fixed by selection. Such birds bred together, give many plain heads and birds with coarse crests."

Bateson also speaks of the relation of other phenomena to the Mendelian conception, such as *continuous* or *discontinuous variation*, *prepotency and the determination of sex*, but it will not be necessary in this connection to quote more at length.

Although the foregoing account of the relation of the history of hybridizing to that of the other biological sciences is from necessity an outline only, I think it can be seen that hybridization has at various times played an important rôle, and occasionally a definitive one, in aiding the solution of perplexing biological problems.

THE RESULTS OF EXPERIMENTAL HYBRIDIZATION

Florists and gardeners have long employed the crossing of closely related plants † as a means of obtaining new and useful races and it is without doubt largely due to them that so much is at present known about the behavior of plant hybrids. To the practical as well as to the theoretical hybridizer the manner of the transmission of the parental characters, the fertility or sterility of the hybrid, and the variation of the hybrid race are of the highest importance.

The variation in hybrids is dependent on the manner in which they reproduce parental characters. These characters may be blended in the hybrid, they may be associated in a variety of ways, or the hybrid may recapitulate in detail the characters of either parent. Moreover, the hybrid of the primary cross may show variation to a different degree from that of the second, or of the later generations. The phenemona in the first generation are the essentials of what is commonly understood by the term hybrid, and as such are described in all literature of the subject; the behavior of the hybrids in the second and following generations, although of the greatest importance, has been little studied.

* By "heterozygote" is evidently meant a hybrid, apparently only dominant, but really with both opposing characters ("allelomorphs" of Bateson, "Paarlings" of Correns) united to form the "zygote"; in the "homozygotes," on the other hand the characters of the pair are alike.

† Compare Richard Bradley's "New Improvements of Planting and Gardening, both Philosophical and Practical," p. 16. London, 1726.

The variations of the hybrids are many and complex, and it would be impossible as well as unnecessary for the purposes of this paper to attempt to present anything like a full account of the subject, much less to give a very complete list of hybrids to illustrate the variations. What I shall give is largely drawn from the work of Focke already quoted, and a shorter one by Swingle and Webber,* although I shall not confine myself wholly to these sources.

The characters of the parents may be perfectly blended in the hybrid so that neither parent is "favored" (*Datura Metel* × *D. meteloides*, *Geum intermedium* †). In other cases the hybrid may not be intermediate but may exhibit all grades of the characters by which the parents are distinguished from each other (*Abutilon* hybrids), even reproducing in great detail either parent (*Hieracium* hybrids, those of the maize, *Cytisus Adami* ‡ and the pods of some hybrid peas, etc. §). Or the hybrid may show the characters of either parent without intermediate grades (strawberry hybrids). The foregoing refers to the behavior of the hybrids of the first generation, or those of the primary cross; in the second generation the variations are equally well marked.

In the second generation the hybrids that were intermediate and uniform in the first one may remain so (*Geum intermedium*, Burbank's raspberry-dewberry hybrid). Or such hybrids may vary (*Nicotiana rustica* × *N. paniculata*). Hybrids which present variations in the first generation tend to become more variable in the second and third generations. Those that in the first generation resembled either parent may continue to revert in a similar manner (strawberry hybrids), or an intermediate condition may be present in addition to the extreme racial forms (*Datura Metel* × *D. meteloides*).

Another phase of variation is found in the changes that a hybrid may exhibit in the course of its life, or ontogeny. In the earlier stages it may show the characters of one parent, and in

* Swingle & Webber, Hybrids and their Utilization in Plant Breeding. Yearb. U. S. Dep. Agric. 1897: 383. 1898.

† Salter, On the Fertility of certain Hybrids. Phytologist, 4: 737. 1852.

‡ Farmer. Ann. Bot. 11: 538. 1897.

§ Tschermak, Weitere Beiträge über Verschiedenwerthigkeit der Merkmale bei Kreuzung von Erbsen und Bohnen, Ber. Deuts. Bot. Gesells. 19: 35. 1901.

the later ones those of the other. For example, hybrid *Tropeolum* which was intermediate at the beginning of the season, reverted later to the mother form with intermediate grades * ; in spring the leaves of hybrid *Cistus* and *Populus* may show characters of one of the parents, and in the autumn those of the other ; or in other hybrids (*Melandrium album* × *M. rubrum*, *Epilobium roscum* × *E. montanum*) the flowers may change during the blossoming from the characters of one parent to those of the other ; or this may take place in different years (*Bletia crispa* × *B. cinnabarina*, *Galium cinereum* × *G. verum*).

In another sort of hybrids the parental characters may reappear side by side — these are the so-called "mosaic" hybrids. They usually result from the union of closely related parents. As a single example of mosaic plant hybrids, that produced by crossing *Rhododendron Rhodora* × *R. calendulaceum* may be cited. In this case the flower has the colors of the flowers of both parents, and these are reproduced unchanged and stand side by side. Darwin † gives an example of an interesting mosaic animal hybrid. He says "the hairless condition of the Paraguay dog is either perfectly or not at all transmitted to its mongrel offspring ; but I have seen one partial exception in a dog of this parentage which had part of its skin hairy, and part naked ; the parts being distinctly separated as in a piebald animal."

Occasionally the hybrids are invariable and come true to seed. Such a one is the *Oenothera* hybrid of de Vries,‡ formed by the union of *O. rubrinervis* and *O. nanella*, which has been grown several generations without reversion. Another case may be mentioned out of many, namely, that of the *Geum* hybrid already referred to. This plant is a natural hybrid in certain parts of England, and was formerly supposed to be a distinct species. Salter § reproduced the hybrid by crossing *G. rivale* and *G. urbanum*. All of these hybrids were uniform and intermediate, and came true to seed for many generations.

Still another class of inheritable characteristics may well be mentioned ; I refer to the transmission of such characters as hardi-

* Darwin, Animals and Plants Under Domestication, Am. ed., 1 : 470. 1868.
† Darwin, *l. c.* 2 : 117.
‡ De Vries, Die Mutationstheorie, 1 : 461. 1901.
§ Salter, *l. c.* 739.

ness, or the reverse of this, the ability to withstand warmer climates. Of the former the hybrid between *Montbretia Pottsii* and *Tritonia aurea* is given by Swingle and Webber (*l. c.* 414), who also present the Le Conte and Kieffer pears as examples of the latter. These writers say (*l. c.* 416) that the "adaptability of the Kieffer and Le Conte pears to growth in warmer climates is doubtless derived from the mother," that is, the Chinese sand pear. The ability to resist diseases is another transmissible character. The French varieties of grapes have been made resistant to phylloxera by crossing with hardy American forms. The hybrid may also be different from either parent in qualities analogous to the above. For example, it may be more vigorous than either parent, or may attain a larger size. Such variations are found in the offspring of closely related plants. The walnut hybrid of Burbank (*Juglans regia* × *J. Californica*), mentioned by Swingle and Webber, "grows twice as fast as the combined growth of both parents. * * * The wood is very compact, with lustrous, silky grain, taking a beautiful polish, and as the annual layers of growth are an inch or more in thickness and the medullary rays prominent, the effect is unique."

We shall take up now briefly the relative sterility or fertility of hybrids, and what is closely associated with it, the limits of a cross.

Focke * says that, as a general rule, "je näher die morphologische und systematische Verwandtschaft der Stammformen ist, um so weniger pflegt das geschlechliche Fortpflanzungsvermögen der Mischlinge von der Norm abzuweichen; je ferner die Stammformen einander stehen, um so mehr zeigt sich durchschnittlich die Fruchtbarkeit der Mischlinge geschwächt."

Increased or total sterility is brought about in various ways, as for example, there may be no pollen and no good seeds produced, or there may be a smaller amount of either than in the pure parents. Focke says that no peculiarity of hybrids has attracted so much attention as the lessening of the power of sexual reproduction, and it has been pointed out how important this fact was formerly supposed to be in its relation to the limits of species and varieties. Darwin (*l. c.* **2**: 218) says that "the sterility of distinct

* Focke, Die Pflanzen-Mischlinge, 489. 1881.

species * * * and that of their hybrid offspring, graduates, by an almost infinite number of steps, from zero * * * up to complete fertility." Focke (*l. c.* 477) gives genera or families that illustrate the variation of plants as regards the fertility of their hybrid offspring. The hybrids of *Papaver*, *Viola*, *Verbascum* and *Digitalis* are slightly fruitful; those of *Anemone*, *Nicotiana*, *Mentha*, *Crinum*, the Cucurbitaceae and the Passifloraceae are often so; while in the following there are more fruitful than unfruitful hybrids: *Aquilegia*, *Dianthus*, *Pelargonium*, *Geum*, *Epilobium*, *Fuchsia*, *Cotyledon*, *Begonia*, *Cirsium*, *Erica*, *Rhododendron*, *Calceolaria*, *Quercus*, *Salix*, *Gladiolus*, *Hippeastrum*, the Gesneriaceae and the Orchidaceae. While the crosses between distinct species produce hybrid progeny that are variable as regards the quality of fertility, it seems that nearly all hybrids from plants more distantly related are wholly sterile. As an example of fertile bigeneric hybrids, however, the well-known Aegilops wheat may be cited; but this rule is apparently invariable as regards the few that have parents from different families.

When the abnormal condition of the male reproductive organs causes sterility, as is likely to be the case, this infertility may be brought about by a variety of structural causes. The anthers may contain pollen but dehiscence may not take place to allow the pollen means of escaping, or pollen may be entirely wanting. In speaking of this question Focke (*l. c.* 478) says that "in andern Fällen besteht der Blüthenstaub aus kleinen pulverigen bei Anfeuchtung nicht quellenden Körnern von ungleicher Form und Grösse, denen gewönlich einzelne wohlgebildete keimfähige Pollenzellen beigemischt sind." The conclusion in this case is that the abnormal pollen is the cause of sterility and that the normal only is functional. Also, according to Duchartre * Naudin made the observation that the relative fertility of hybrids was *en rapport* with the number of normal pollen-grains formed in the hybrid.

Another and much less usual cause of sterility is found in the apparent incapacity of the female organs to be fecundated, but in many such cases it may be that there is no morphological defect in the ovule. Such a hybrid may sometimes, or usually, be fruitful if fertilized by another plant of the same strain, or by one of the parents, and it is occasionally fertile when self-fertilized, or

* Duchartre. Ann. Sci. Nat. Bot. IV. 19: 129. 1863.

when pollinated by a plant of the same sort (*Rubus caesius* × *R. Idaeus*).

Besides the forms of sterility that result from imperfect sexual organs an infertile condition may also result from other causes. For instance, in unisexual flowers, the male buds may fall away, as in the Cucurbitaceae and the Bignoniaceae, or in some hybrids, *Pelargonium* and *Digitalis* (*D. lutea* × *D. purpurea tubiflora* Lindl.), the anthers may be so transformed that the flowers are hermaphrodite. Finally, sterility may manifest itself in the reluctance with which hybrids produce flowers, as in those of *Rhododendron*, *Epilobium*, *Cereus* and *Hymenocallis*, but Focke (*l. c.* 477) says that these are rare exceptions, since as a rule hybrids flower earlier and more profusely than pure species.

As to the relation between vegetative vigor and relative fruitfulness of hybrids, Swingle and Webber (*l. c.* 412) say that in some cases "the increase in vegetative vigor secured by crossing distinct species is at the expense of fertility, but this is by no means true in all. Focke says that 'it was formerly thought that the diminished sexual fruitfulness is compensated by a greater vegetative luxuriance, a statement the untenableness of which, as Gärtner showed, is most plainly demonstrated by the fact that many of the most fruitful crosses (*Datura*, *Mirabilis*) are also distinguished by a most gigantic growth.' On this subject Fritz Müller also says: 'So far as my experience goes, the hybrids which grow the most luxuriantly are generally the most fruitful.'" Although other instances illustrating this principle might be given it will suffice, I think, to mention the remarkable walnut hybrid which Burbank succeeded in producing from the English form and the California one. This was referred to above (page 144). In this hybrid not only is there a great vigor of growth but the fruit is also unusually large and probably very abundant.

There appears to be no relation between the ease of making a cross and the fertility of the resulting form, but as a rule, the nearer the relationship of the parental forms the more readily will they be crossed. Strasburger,* in commenting on this subject, says: "Manche Familien neigen leichter dazu (Solanaceen, Caryophyllaceen, Irideen u. s. w.), andere bilden nur schwierig oder

* Strasburger, Lehrbuch der Botanik, Ed. 5, 248. 1902.

überhaupt keine Bastarde (Papilionaceen, Coniferen, Urticaceen, Convolvulaceen u. s. w.). Dasselbe abweichende Verhalten findet sich unter verwandten Gattungen und Arten vor. Weinreben, Weiden, *Dianthus*-Arten sind leicht, *Silene*-Arten schwer, die von *Nicotiana*, *Verbascum*, *Geum* leicht, die Arten von *Solanum*, *Linaria*, *Potentilla* dagegen schwer unter einander zu bastardiren. Eine Hybridisirung von nahe verwandten Arten will oft nicht gelingen (z. B. Apfel- und Birnbaum), dagegen lassen sich Pfirsich mit Mandel, ja sogar Species der verschiedenen Gattungen *Lychnis* und *Silene*, *Rhododendron* und *Azalea*, *Aegilops* und *Triticum* kreuzen, je nach ihrer 'sexuellen Affinität.'"

The suggestion of Strasburger that crosses between mosses and ferns cannot take place because of the difference in the chemical nature of the fluids attractive to the spermatozoids of the two forms, may be a fruitful one when applied to other plants that are difficult or impossible to cross. That is, the substance that attracts spermatozoids, or the pollen-tube, may vary with the plant, and instead of attracting may even repel them. So far as I know the course of the pollen-tube in plants that are nearly enough related for crossing, but which refuse to be crossed, has not been traced, and it is possible that here may be found one reason why certain reciprocal crosses will not take place, why nearly related plants may sometimes not be crossed, and also why one sort of pollen is "prepotent" over another sort. That the pollen-tube responds to such stimulus in the course of its wanderings most sensitively is well shown by Lloyd's account* of its behavior in *Diodia* and *Richardsonia*. There may be other factors, as Vernon† has found that the relative maturity of the sexual elements in some echinoderms is of great importance when the number of successful crosses, and even the effect of each parent on the offspring, are concerned.

While it is perfectly true that only nearly related forms as a rule may be crossed, there are many instances where hybrids have been made whose parents were generically and even more remotely separated. These, as was mentioned above, are usually wholly sterile. A few such hybrids may be mentioned here.

* Lloyd. Mem. Torrey Club, 8: 88. 1902.

† Vernon, Hybrid Echinoid Larvae. Proc. Roy. Soc. 63: 228. 1898.

Gladiolus blandus Sol. of the lily family was fertilized with pollen of *Hippeastrum* sp. and seeds were formed that produced four hybrids, all of which were presumably sterile (Focke, *l. c.* 388). The same author says that *Digitalis ambigua* of the Scrophulariaceae was crossed by Campbell with pollen of *Sinningia speciosa* of the Gloxiniaceae, and of the seeds that were formed only one produced a plant, and that one was sterile. The limits for any cross are thus set by related families, but as has already been shown the limits bounded by the fertility of the offspring are much narrower.

STUDIES OF THE SPERMATOGENESIS AND STRUCTURE OF HYBRIDS

A few morphological studies of hybrids have been made which may now be briefly referred to. I shall speak first of the work on the gametogenesis of hybrids.

Juel * studied the spermatogenesis of *Syringa Rothomagensis*, and as a leading result found that the maturation mitoses were irregular and abnormal, although in some respects certain of the mitoses reported by him approach the usual or normal type. The abnormalities in the tetrad formation are classified and summarized by him as follows:

A. In früheren Entwickelungsstadien auftretend:
   1. Verkümmern der Pollenmutterzellen beim Eintritt des Spiremstadiums;
   2. Durchschnürung des Kernes der Pollenmutterzelle im Spiremstadium;

B. Bei der Tetradentheilung auftretend:
   3. Durchschnürung der Kerne;
   4. Erste Kerntheilung indirect, aber in Bezug auf das Verhalten der Chromosomen abnorm;
   5. Achromatische Kernfigur abnorm;
   6. Ein Theil des Chromatins im Cytoplasma zerstreut;

C. An den Tetraden beobachtet:
   7. Ueberzählige Tetraden. Wahrscheinlich aus 2 hervorgegangen.
   8. Ueberschüssige Kerne in den Zellen der Tetrade. Wahrscheinlich aus 6 hervorgegangen.

* Juel, Beiträge zur Kenntniss der Tetradentheilung. 2. Die Tetradentheilung bei einer hybriden Pflanze. Jahrb. Wiss. Bot. 35: 638. 1900.

I wish to call attention especially to number 6 of the above table, namely, to that form of irregularity in the distribution of the chromosomes by which a portion of them are thrown out of the nucleus, a portion only remaining. The result of this might be, as Juel suggests, that the nucleus of the tetrad would contain chromatin of pure descent, that is, chromatin derived from one and not from both parents.

Guyer * has studied the spermatogenesis of hybrid pigeons and of hybrid cannas and found in both cannas and pigeons similar mitoses; consequently it will be necessary to speak only of the mitoses in the hybrid pigeon. I shall quote direct (*l. c.* 312):

"In the spermatogenesis of hybrid pigeons several abnormalities are manifested. These may be classified conveniently under three heads: (1) abnormalities in the structure of the spermatozoa; (2) abnormalities in mitoses; (3) degeneration of the germinal cells. Abnormalities in the spermatozoan structure were present in sterile hybrids, the most noticeable feature being a varicosity or swelling about the middle of the head. In tracing the development of the spermatozoa, this curious modification was found to be due apparently to a lack of development of the head; the nucleus did not elongate completely as in normal spermatogenesis. Abnormalities in mitosis were marked in both fertile and sterile hybrids. Large numbers of multipolar spindles were present. These were usually of the tripolar type. Occasionally two distinct and separate spindles occurred in one cell. The spermatocytes of the first order were the cells that showed this phenomenon to the greatest extent. In the normal pigeon the chromosomes in the spermatogonia are sixteen in number and in the primary spermatocyte eight. The latter are laid down in rings and each is evidently double. On the spermatogonia of the hybrid there were sixteen chromosomes and in the primary spermatocyte often more than eight. In the latter there may be several of the large double type and a number of smaller rings, or sixteen small ring chromosomes may occur. If sixteen rings were present they were usually located on two separate spindles, eight to each spindle.

* Guyer, Spermatogenesis in Hybrid Pigeons. Science, II. 11: 248, 312. 1900; Some Notes on Hybridism, Variation and Irregularities in the Division of the Germ-cell. Science. II. 15: 530. 1902.

Another peculiarity in the mitosis was the frequent inequality in the division of the chromosomes, in some instances only about one fourth of a chromosome going to one pole." At page 248 he concludes that "these peculiarities in chromosome formation may point perhaps to a tendency in the chromatin of each parent species to retain its individuality. If such is the case, then in those cells with two spindles each bearing eight chromosomes, it is evident that after division, some of the new cells will have chromatin from only one of the original parent species, and some from the other. Some of the spermatozoa, therefore, will bear chromatin from only one of these species. It is a well-known fact that the offspring of hybrids are extremely variable, a portion of these variations being usually in the form of reversions to one or the other of the parent species. The possibility presents itself then, that this reversion may be due to the persistence of the chromatin of only one species in one or both of the germ cells. Carrying the conception still further, the other variations in the offspring of hybrids may be due, perhaps, to the varying proportions of the chromatin of each species in the mature germ cells."

And, finally, Metcalf* has studied the spermatogenesis of hybrid *Gladiolus*. He finds a condition analogous to the two-spindle nuclei of hybrid pigeons in all of the pollen-mother-cells of the hybrid, and he also concludes from this that the two groups of chromosomes represent maternal and paternal derivatives respectively. It is not stated whether the hybrid is a fertile one.†

In addition to the cytological studies as above outlined, some work on the structure of hybrids has also been done. This may be summarized in the following paragraphs.

Macfarlane‡ conducted an extensive series of investigations on the minute anatomy of several hybrids, his main result being that all of the hybrids which he studied were intermediate in structure,

* Metcalf, Certain Problems Relating to the Individuality of Chromosomes. Proc. Neb. Acad. Sci. 7 : 109. N 1901 (read before the Academy, N 1900).

† Metcalf in a private letter assures me that the hybrid *Gladiolus* is fertile.

‡ Macfarlane, The Microscopic Structure of Hybrids. Gard. Chron. III. 7 : 543. 1890; A Comparison of the minute Structure of Plant Hybrids with that of their Parents, and its bearing on biological problems. Trans. Roy. Soc. Edinb. 37^1 : 203. 1892; Observations on Pitchered Insectivorous Plants. Ann. Bot. 7 : 445. 1893; Observations on some Hybrids between *Drosera filiformis* and *D. intermedia*. Contr. Bot. Lab. Univ. Penn. 2 : 87. 1898.

even in the details, and his conclusion that the parents share equally in transmitting their respective characters.

Farmer * studied the structure of *Polypodium Schneideri*, which is a hybrid between *P. aureum* and *P. vulgare*. The hybrid was reproduced by Farmer. The study showed the hybrid was not strictly intermediate either in form or structure.

In the following section of this paper I shall consider the relation of the results of the cytological studies of hybrids as above outlined, as well as of my own to be given later, to the variation of the hybrid race, but I may refer in this place to one or two considerations in connection with the possible bearing of the structural studies on the same topic. It is to be noted that Macfarlane studied only intermediate forms, but on the other hand the fern hybrid of Farmer's investigations was intermediate neither in form nor in structure. These apparently contradictory results raise the very interesting question, are hybrids, whether intermediate in form or not, always so in structure? Or can it be that variation in form is also associated with differences in structure? A careful histological study of hybrids that are clearly not intermediate, as for instance those following Mendel's *Pisum*-type could not fail to give most interesting results. And it may be added that the results from such study might be of importance in determining the hybrid status of the form.

THE RELATION OF THE CYTOLOGICAL TO THE EXPERIMENTAL STUDIES OF HYBRIDS

The task of the cytologist in the study of plant hybrids is primarily that of observing the relation of the manner of formation of the sex-cells to the variation of the hybrid itself, and it should for that reason include the study of hybrids which have yielded definite results from experiment, such forms, for example, as the *Pisum*-type. Owing however to the recentness of the discovery of the Mendelian laws, studies of this character have not been carried on, as far as I know, and conclusions to cover special cases of variation, for instance the *Pisum*-type, must therefore be regarded as tentative merely. Beyond this, general considerations, such as the fertility or infertility of the hybrid and the form

* Farmer, On the Structure of a Hybrid Fern. Ann. Bot. 11: 533. 1897.

of nuclear division in it, may give grounds for interesting and legitimate conclusions. It is of course only these general considerations that I can take up in the present paper.

From the summary of Juel's work on the *Syringa* hybrid it was seen that all of the maturation divisions were more or less abnormal. What may perhaps be considered the nearest approach to the normal division observed in this hybrid was an apparently typical achromatic figure accompanied by many small chromosomes, which were therefore not like those of heterotypic nuclear mitoses, and which did not split in the metaphase. And it is of interest to note that *Syringa* is probably a sterile hybrid.

Turning now to the results of Guyer from the study of fertile hybrid pigeons, we note that he observed both normal and abnormal mitoses, and some mitoses which were not clearly the one or the other. The latter are of particular interest since it is primarily these that he considers a possible basis for the variation of hybrids.* Among the irregularities in the mitoses in fertile birds, which may contribute to variation, is the following. In the dividing nuclei of some secondary spermatocytes two spindles occur, each bearing half the somatic number of small chromosomes.† As previously given, Guyer concludes (1) that the two-spindle condition shows a tendency in the chromatin of each parent to retain its individuality, (2) that by a proper separation and distribution of the chromosomes pure sex-cells would result, and (3) that the union of these might cause reversion. This conclusion appears to have been drawn before the publication of the Mendelian law.‡

If the form of maturation mitosis just described occurs generally in hybrids, and further if the chromatin is separated and distributed as postulated by Guyer, we should evidently not have to go further in order to discover the kind of mitosis that would produce in hybrids of the *Pisum*-type "pure" germ-cells — the important part of the Mendelian theory.§ There are however one or

* Guyer. Science, II. 11: 248. 1900.

† Compare Metcalf on hybrid *Gladiolus*, *l. c.*

‡ In his most recent paper (see footnote on p. 155) which was received after this was written, Guyer considers it possible that "pure" reproductive cells may be derived from normal mitoses.

§ Bateson and Saunders, *l. c.* 12: "the essential part of the discovery is that *the germ-cells or gametes produced by cross-bred organisms may in respect of given characters be of the pure parental types.*"

two considerations in this connection which may be worth bringing forward. In the first place the possibility arises that the spores that are formed from two-spindle nuclei in the mother-cells, even if functional, may contain chromatin from both and not from one parent only. To put this idea somewhat more directly, it may be that the failure of the maternal chromatin to unite with the paternal chromatin, which will be spoken of again presently, results not in the purity of the reproductive cells, but quite the contrary, in their being of hybrid nature. This has certain theoretical interest and will be again referred to. In addition to this the results of my own study of the spermatogenesis of hybrid cotton, which will be given in the following section, lead me to believe that the usual form of maturation division in fertile hybrids is quite like that in the pure race (as indeed Guyer also found in fertile pigeon hybrids), and therefore that the variations in the hybrid cotton are not dependent on irregularities in the mitoses, and consequently if irregularities occur it must be shown that the resulting spores are functional. (The possible exception to this will be given below.)

Now since all of the hybrids known to be fertile which have thus far been studied have perfectly normal maturation mitoses, in addition to clearly irregular ones, and further since in known sterile forms abnormal divisions only occur, it seems to me that the variations of the hybrids are either wholly independent of those divisions, or that the mitoses are fundamentally unlike what is now believed. Correns * has apparently reached the former conclusion; he does not think that irregularities in the maturation mitoses have any connection with the splitting of the hybrid race.

It now remains to be seen what sort of maturation mitoses there may be (1) which will result in the separation and distribution of the chromatin so that the ultimate sex-cells may have chromatin of pure descent, and (2) which will also appear to be the same as is believed to occur in pure forms, namely, a double longitudinal division of the chromatin segments.

I shall now refer to the account given by Wilson † of the formation of tetrads by conjugation, but more especially to his ac-

* Correns, Ergebnisse der neusten Bastardforschungen für die Vererbungslehre. Ber. Deuts. Bot. Gesells. 19: 86. 1901.

† Wilson, The Cell, ed. 2. 1900.

count of the early history of the germ-nuclei. In speaking of the latter, Wilson says (*l. c.* 273): "A large number of observers are now agreed that during the growth-period preceding the maturation division, in both sexes, the nucleus of the mother-cell (spermatogonium, oögonium), both in plants and in animals, passes through some changes preparatory to reduction at a very early period. Thus, in the egg the primary chromatin-rods are often present in the very young ovarian eggs, and from their first appearance are already split longitudinally. Häcker made the interesting discovery that in some of the copepods (*Canthocamptus*, *Cyclops*) these double rods could be traced back continuously to a double spireme-thread, following immediately upon the division of the last generation of oögonia, and that *at no period is a true reticulum formed in the germinal vesicle.* In the following year Rückert made a precisely similar discovery in the case of selachians. After division of the last generation of oögonia the daughter-chromosomes do not give rise to a reticulum, but split lengthwise, and persist in this condition throughout the entire growth-period of the egg. Rückert therefore concluded that the germinal vesicle of the selachians is to be regarded as a 'daughter-spireme of the oögonium (*Ur-ei*) grown to enormous dimensions, the chromosomes of which are doubled and arranged in pairs.' In this case their number seems to be at first the somatic number (thirty-six) which is afterward halved by the conjugation of the elements two and two (Rückert), as in *Lumbricus* (Calkins). It is, however, certain that in many cases (insects, copepods) the double rods first appear in the reduced number." In another place (*l. c.* 257), Wilson says: "A considerable number of observers have maintained that reduction may be effected by the union or conjugation of chromosomes previously separate. This view agrees in principle with that of Rückert, Häcker and Vom Rath; for bivalent chromosomes assumed by these authors may be conceived as two conjugated chromosomes." It should be added further that Montgomery * and evidently Guyer as well † consider the conjugating chromosomes to be of unlike parentage. Therefore the hetero-

* Montgomery, A Study of the Chromosomes of the Germ Cells of the Metazoa. Trans. Am. Phil. Soc. 20: 1901.

† Guyer. Science. II. 15: 530. 1902.

type rings of the normal mitoses in pure races of animals are not only of double origin, but they are so organized that the halves of any chromatin loop are of diverse origin, that is, one half is derived from the one and the other half from the other parent. So that in pure races of animals *if the chromatin is of pure descent*, and, further, *if the maternal and paternal chromatin is segregated as a result of the maturation mitoses*, the ultimate sex-cells are also of pure descent, two of any group containing chromatin lineally derived from one parent and two chromatin from the other. I therefore have suggested* that the same organization of the chromatin occurs in fertile hybrids, and that it forms the morphological basis for variation in accord with the Mendelian conception.†

The organization of heterotype rings by the conjugation two and two of previously distinct chromosomes appears thus to be fairly well founded, but the purity of the chromosomes as regards descent is, I take it, not so well established, and here may perhaps be sought a cause for variations outside of the *Pisum*-type. But there is also considerable evidence to show that the chromosomes preserve their individuality during the ontogeny, or if the chromosomes do not retain their individualty, at any rate that the chromatin may retain its purity. Wilson (*l. c.* 204) says: "Observations have given the strongest reason to believe that, as far as the chromatin is concerned, a true fusion of the nuclei never takes place during fertilization, and that the paternal and maternal chromatin *may* remain separate and distinct in the later stages of development—possibly throughout life." On page 299 this

*Cannon, A Cytological Basis for the Mendelian Laws. Bull. Torrey Club, 29: 657. D 1902.

†This conclusion was also independently formed by W. S. Sutton: On the Morphology of the Chromosome Group in *Brachystola magna*. Biol. Bull. 4: 24. D 1902. Guyer has recently advanced a similar conclusion: Hybridism and the Germ-cell. Univ. Cincinnati Bull. No. 21. N 1902.

The hypothesis above given to account for the variation of the hybrid race after the type of Mendel's pea hybrids applies of course to those whose pure parents are separated by one character only, that is, to monohybrids, and does not clearly to polyhybrids, or to those whose pure parents are distinguished by more than one character. If, however, we find that the two-spindle condition obtains in the spore-mother-cells of fertile hybrids to a considerable degree it seems to me that in the combined results of such abnormal with the normal mitoses we may find a structural basis for the essentials of the variations in the more complex types of hybrids.

author treats the matter somewhat more fully and cites the work of Rükert and Häcker on *Cyclops*, and Herla and Zoja on *Ascaris*. Recently Häcker * has confirmed and extended his earlier conclusions, and in a recent paper Moenkhaus states that he has found that the paternal and the maternal chromatin may remain separate and distinct during several cell generations in hybrid fishes.

It has already been stated that the conjugation of the chromosomes occurs in pure races at some stage before the maturation divisions, and that by the separation of the chromosomes, provided they are lineally descended from parental chromosomes, the spores may be of pure descent. Now when in certain hybrids the conjugation fails to take place a mother-cell with a two-spindle nucleus may be formed as Guyer has stated, † and as he has shown in the fertile hybrids studied by him. Metcalf also found in hybrid *Gladiolus*, as I have mentioned in summarizing his work, chromosomes in two masses as on double spindles in the pollen-mother-cells and, finally, I have observed a few mother-cells in the hybrid cotton which may apparently had double spindles. In the cotton, however, this condition was the exception, as will be spoken of below.

Phenomena which are probably associated with the independence of the germ-nuclei have long been observed in hybrids. These comprise the greater influence of the one than the other parent, or, at the other extreme, the equal influence of both parents in the hybrid. Between these extremes are many intermediates some of which have already been mentioned (page 142). And it seems possible that in some hybrids the germ-nuclei may equally and independently mold the character of the cells, and not combine to effect this as in strictly intermediate hybrids. Farmer,‡ for example, gives an account of a hybrid *Oxalis* in some epidermal cells of which *trichomes typical of both parents* may be found in a single cell. Other cases illustrating this point might also be cited. It will thus be seen that a close study of the struc-

* Häcker, Ueber die Autonomie der väterlichen und mütterlichen Kernsubstanz vom Ei bis zu den Fortpflanzungszellen. Anat. Anzeiger, 20: 440–452. 1902.

† Guyer. Science. II. 15: 530. 1902.

‡ Farmer. Ann. Bot. 11: 542. 1897.

ture of a hybrid may be of considerable use in determining the relative independence of the germ-nuclei.

I wish now to turn from the considerations of nuclear divisions and hybrid variations and apply *theoretically* the principle of the purity of the sex-cells to a moss, a fern, and a seed-plant. In doing so I assume, as a matter of course, that the hypothetical hybrids are monohybrids of the *Pisum*-type. The theoretical application is made rather to call attention to what seems to me a very broad and inviting field for research, than to attempt at this time any development of the field myself. Many additional points of view will undoubtedly suggest themselves at once to any botanist.

We shall consider what happens when one sort of a moss is crossed with another sort, when the spores of the hybrid moss develop, and, finally, when the hybrid is inbred.

In the first place when a moss is fertilized by the sperms of a moss of another species, the moss "plant" is not hybridized, since the effect of the foreign fertilization is to be observed only in the *sporogonium*. This applies to the hybrid of the first generation; what happens in the later generations has apparently not been studied or at least recorded. Focke (*l. c.* 427) says that no hybrid sexual moss plants (gametophytes) have been recognized, and he inquires if some of the numerous sterile mosses may not be hybrid. According to the hypothesis of the purity of the spores we should not expect to find hybrid gametophytes in any generation. And in this connection it is certainly interesting to note that there is said to be no hybrid among the liverworts. This again would be the case if the spores from which the liverwort "plant" arises are not hybrid but pure, and we may perhaps make the rule that *it is the sporophyte, and not the gametophyte, which is of hybrid nature.*

Assuming the purity of the spores, when the hybrid is inbred variation of the sporogonia would occur according to the law of Mendel, but not necessarily according to the *Pisum*-type. The variation of the hybrid will depend on the monoecious or dioecious nature of the moss. This will be clear when we examine the formulae for variation of monoecious and of dioecious ferns.

Turning now to the fern hybrids, we shall first consider a monoecious homosporous hybrid, and, finally, a dioecious homosporous hybrid.

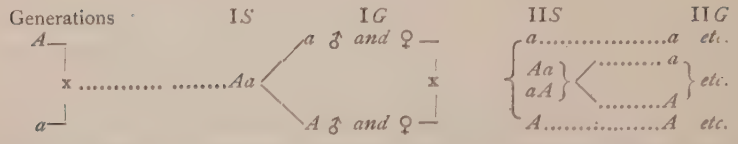

FIG. 2. Hybrid homosporous *monoecious* fern. Explanation. *S*, sporophyte; *G*, gametophyte; *A* and *a*, the chromosomes of the two races, when combined (*A a*) the form is hybrid.

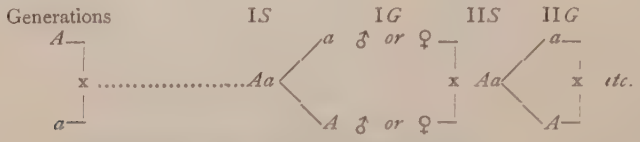

FIG. 3. Hybrid homosporous *dioecious* fern. *Lettered as the preceding figure.*

In contrasting the two formulae for fern hybrids as given several points may be worth speaking of. In the first place the fern hybrids with monoecious prothallia revert after the *Pisum*-type of Mendel; and secondly the hybrids with dioecious sexual generations are always some form of intermediate, and never revert. Dioecious forms also, such as *Equisetum* hybrids, suggest problems of interest not necessarily closely connected with their hybridity. Among these the following may be mentioned: Dioecious ferns viewed from the standpoint of the purity of the spores, and hence of the sex cells, give suggestions as to the morphological basis of the origin of heterospory. Coulter * says: "The evolution of heterospory seems simple enough. The physiological differentiation of the spores was complete when prothallia became persistently dioecious. * * * A prothallium producing both sex organs equally well may be regarded as in a state of equilibrium, an equilibrium which is disturbed by any conditions which favor the production of one sex organ rather than the other, in this case probably nutritive conditions. This disturbance of the equilibrium of a bisexual prothallium would certainly find an expression first in a dioecious tendency, and finally in a dioecious habit. With the habit once fixed the morphological differentiation of spores becomes inevitable, since the nutritive requirements of the two prothallia are so different. The evolution of heterospory seems to be one of the simplest of selective processes, with inequalities of nutrition to furnish the variations."

* Coulter. Bot. Gaz. 26: 163. 1898.

I would suggest that the transmission of a *dioecious tendency* is morphologically possible because the spores that give rise to the prothallia are pure, and, therefore, we conclude, tend to reproduce the same sort of prothallia as the ones from which they sprang in the preceding generation, and that the *dioecious habit* is likewise only possible by a continuance of the same process through many generations. By referring to Fig. 3, the formula for a dioecious fern hybrid, it is seen first that the gametophytes are of pure descent, and further that the fern is dioecious, so I suggest that the spores *A* will produce prothallia like those from which the chromatin of their nuclei was derived and, also, that the spores *a* will give rise to prothallia like *a* of the pure form, that is, in *dioecious hybrid ferns, or the pure races, the prothallia that arise from A or a will be male or female accordingly as the sexual ancestor of that spore was male or female.* In other words, may not the purity of the spores be the groundwork upon which the selective processes play that bring about the dioecious from the monoecious condition, that deepen the dioecious tendency into a dioecious habit, and, finally, give rise to the morphological condition known as heterospory?

We may now return from this disgression to examine very briefly what happens when seed-plants of different sorts are crossed.

We should note first that the formula commonly given for the hybrid fertilization and variation in seed-plants is somewhat misleading since it does not take account of the sexual generation, which we believe in the monohybrid seed-plants of the *Pisum*-type, if indeed not in all others, is always pure. Secondly, it is the heterosporous condition of the higher plants, in the place of the homosporous monoecious one of the fern mentioned above, that makes possible the formula ($A + A$; $A + a$; $a + A$; $a + a$) of the hybrid of the *Pisum*-type. Now we may ask, what morphological conditions are prerequisite for the development of this formula? It is clear in order that this end may be reached that the anthers must organize spores with *A* and spores with *a* characters and likewise that the ovary must maturate functional spores with *A* and with *a* characters. Now as a matter of fact in the higher plants three of the potential macrospores usually degenerate and one only germinates, so that in any ovule one character, and one sort of chromatin, are eliminated from the problem. Therefore

the reversion to one or to another of the pure parents might not under certain conditions take place. It thus is necessary in order to effect the splitting after the *Pisum*-type that on the average, taking many plants together, an equal number of each sort of germ-cells of the same descent shall degenerate, and that a like number shall become functional. The variability of the higher plants as regards the development of the macrospores may thus in a broad way account for certain conceivable modifications or apparent exceptions to the Mendelian law, so that the morphology of the hybrids must needs be studied if the structural basis for hybrid variation is to be understood.

We have then sketched what supposedly takes place when mosses, ferns and also when seed-plants of the *Pisum*-type are hybridized, and the general and particular conclusions may be summed up thus:

1. In monohybrids of the *Pisum*-type the spores and the immediate spore derivatives are pure as regards the chromatin content of their nuclei. The application of this principle to the *liverworts* accounts for the apparent lack of hybrids among them, either in the first or in the later generations; applied to the *mosses* it accounts for the failure to recognize hybrids in the second and later generations. In both liverworts and mosses the "plant" (gametophyte) is of pure descent in any generation, and the sporogonia only are hybrid. This last consideration leads to the generalization that in *plants of whatever class where hybridity occurs it is perhaps always the sporophyte only which is hybrid.**

2. In the ferns the chromatin may be transmitted from one sexual generation to another in such a manner as to retain its individuality and thus it may tend to reproduce the sort of gametophyte from which it descended, and in dioecious forms the sex also, so that *the purity of the spores forms the background that may make the genesis and the evolution of heterospory a morphological possibility.*

It is likely that the study of such hybrids as have distinct sexual and asexual generations would be of advantage since in them a morphological analysis of the nature of a hybrid might be made, and thus a clearer understanding of the nature of hybridity

* The possible exceptions to this have already been referred to (page 155).

in general but particularly the nature of hybridity in such forms as do not have distinct sexual and asexual generations might be obtained, that is, the hybrids among the seed-plants.

THE SPERMATOGENESIS OF HYBRID COTTON

The cotton hybrid used in this study * was grown from seed obtained by crossing the Sea Island (Constellation brand) with upland (Klondike brand) cotton, *Gossypium Barbadense* × *G. herbaceum*. The crossing was done expressly for this study by Dr. H. J. Webber, of the Department of Agriculture, in the experimental plots near Columbia, South Carolina. Concerning this cross Dr. Webber writes as follows:

"The flowers were emasculated August 22, 1900, and crossed August 23, the resulting boll producing ten seeds; all of the seed of this we sent to you. But 34*a*, which is of the same parentage and crossed at the same time in our plots at Columbia, all proved to be without question hybrids, being very tall and rank and rather unfruitful, showing in practically every character their hybrid nature." He further says that "34*b* is without much question a true hybrid." The seeds from the cross known as 34*b* were sown in pots in the propagating house at the New York Botanical Garden, about July 20, 1901, and the plants that were derived from these seeds were used by me in this study.

The cotton plants, 34*b*, formed buds in October and the first lot for study was put up on the 29th, and after that at intervals of a week or so as often as the buds appeared to be of the proper size. The hybrids appeared to correspond to the description that Dr. Webber gave for the hybrid of the same parentage, 34*a*, and the 34*b* forms were evidently true hybrids. It was evident also that these hybrids were fertile, but to what degree I was unable to determine, since most of the flowers were removed for study.

Before describing the maturation divisions I may anticipate my results by saying that abnormal sex-cells were present in practically all anthers, and, except in the last flowers formed, normal

* I wish here to acknowledge my obligations to Dr. Webber, who kindly made the crosses for me and always encouraged the work; to Professor Underwood, of Columbia University, for his counsel and assistance, and to the officers of the New York Botanical Garden, especially to Dr. MacDougal, who placed the excellent facilities of the Garden at my disposal.

cells as well. The ratio between the two was an inconstant one; in this regard the cotton is different from the *Gladiolus* hybrid, and agrees with the pigeon and *Canna* hybrids, as will be seen by referring to the summary given above. I may also say that my principal object in making the study was to learn whether the mitoses were different from those in pure races of analogous plants, rather than to make an exhaustive study of the divisions as such, and for this reason my material was not suitable for the study of the earlier stages, however important that may be.

I shall turn now to the morphological part of the paper. The divisions in the normal cells will be spoken of first, and then I shall give some account of the various irregularities which may be seen in the abnormal mitoses of the hybrid.

The archesporial cells arise by the division of subepidermal cells and become pollen mother-cells directly, no formation of sporogonous tissue occurring. After the two divisions of its nucleus, the mother-cell undergoes two simultaneous divisions by which the tetrads are formed, and these probably become pollen grains in the usual manner, although the later history of the cells was not learned. The mitoses leading up to the formation of the tetrads are as usual heterotypic and homotypic, and therefore are the same as are found in like mitoses of analogous organisms.

The cytoplasm of the archesporial cells is of a recticular or alveolar structure, and this structure is uniform throughout the cytoplasm of the cell. That is, the portion of the cytoplasm which is immediately around the nucleus is not differentiated from the portion which is in the more peripheral part, but has a similar appearance and structure. This primitive, or undifferentiated condition of the cytoplasm is retained in all cells in which direct nuclear division occurs, but on the other hand it may not be observed in the normal cells. Thus the structure of the cytoplasm forms an interesting and reliable check to the observations of the older stages of development of the cells.

When those of the archesporial cells which are to develop into normal pollen grains become spore mother-cells, the cytoplasm withdraws from the cell walls. This retreat of the cytoplasm may also be observed in certain of the abnormal cells, for instance, in those which do not undergo degeneration in the earlier stages,

as will be spoken of again in this paper. The withdrawal of the cytoplasm is to be regarded as the normal and regular occurrence in the archesporial cells of cotton, and is not therefore analogous to the condition observed by Juel in *Syringa* * where it was an indication of degeneration.

About the time of the formation of the spore mother-cells, and as a regular structural change accompanying it, the cytoplasm of the normal cells shows a marked change in its structure. A zone of dense protoplasm which takes the cytoplasmic stain with avidity, is formed around the nucleus in contact with the nuclear wall. This perinuclear zone is found also in the developing pollen grains of various plants of pure descent.† It is seen not only in the cytoplasm of the spore mother-cell, but in the later stages as well, even in the young tetrads as in *Lavatera*.‡ The structure of the zone appears to be granular, although in some preparations and under comparatively powerful magnification—Zeiss ocular 18, apochromatic oil-immersion 2-mm. objective—it seemed reticular, thus resembling *Larix*.§ Outside of the perinuclear zone is the main portion of the cytoplasm which is alveolar in structure and in addition to these two sorts of protoplasm a layer of fibrillae midway between the periphery of the cell and the perinuclear zone is also occasionally found in the spore mother-cells. When first observed the fibrillar layer is of a rather loose structure, but in the older cells the fibrillae are closely bound together and form a compact zone.

A similar zone is figured by Mottier for *Lilium*. || This filar plasm, like the achromatic figure, takes the gentian-violet with Flemming's triple stain. As a rule the spore-mother cells do not contain this structure, but it is found with great regularity in the cytoplasm of the older cells. The genesis of the fibrillar layer is difficult to account for in the spore mother-cells; in the later stages of development, however, it is formed by the mantle fibers

* Juel, *l. c.* 640.

† Lloyd. Mem. Torrey Club, 8: 71. 1902.

‡ Byxbee. The Development of the Karyokinetic Spindle in the Pollen-Mother-Cells of *Lavatera*. Proc. Calif. Acad. Bot. III, 2: 63. 1900.

§ Belajeff, Zur Kenntniss der Karyokinese bei den Pflanzen. Flora, 79: 430. 1894.

|| Mottier, Beiträge zur Kenntniss der Kerntheilung in den Pollenmutterzellen etc. Jahrb. Wiss. Bot. 30: *pl. 3. f. 5.* 1897.

which stray into the cytoplasm from the two poles of the spindle.

My material was not suitable for the study of the early history of the chromatin of these cells and therefore the account of the maturation divisions begins with the spore mother-cell and includes the two maturation mitoses by which the tetrads are organized.

The first indication of the approaching first division of the nucleus of the spore mother-cell which was observed was the aggregation of the chromatin to one side of the nucleus and the formation there of the spireme. Thus synapsis occurs before the mother-cell withdraws from the cell-wall (*f. 1*). The spireme ribbon loosens and at length its convolutions extend throughout the nucleus. At first the spireme has a beaded appearance and an uneven outline, but in the older nuclei the contour is much more uniform and the structure much more dense. At the same time the spireme increases in its power of absorbing stains. Particularly in the later stages the splitting of the spireme was observed with great clearness.

When the spireme forms segments, the chromatin for a second time collects to one side of the nucleus, and in that situation the shortening and thickening of the segments takes place. The segments are long and in delicate loops when first clearly made out; the loops are much twisted and bent in a variety of ways. The ends of the loops are not infrequently connected by delicate protoplasmic strands, indicating perhaps that the fusion of the adjacent chromosomes had not been a complete one (*f. 4*), and it also may happen that the union of the chromosomes did not take place exactly at their ends but at a greater or less distance from them. Usually, however, the loops are entire and symmetrical and give no hint as to their possible origin or formation. Very frequently in the long chromatin loops a longitudinal splitting may be seen, which must evidently be regarded as a precocious process looking to a subsequent separation of the divided portions.

The chromatin loops shorten and thicken to form rings and they in turn by further condensation lose the ring character, but in the metaphase of the first division this character reappears.

In any nucleus the loops and the rings are of a uniform size,

that is, the two sizes of rings found in the hybrid pigeon * and in some pure forms in plants † were not observed in the cotton.

At the time when the chromatin segments are becoming condensed and have gathered at one side of the nucleus, characteristic changes are also taking place in the structure of the perinuclear zone of cytoplasm. The inner portion of this becomes less dense and is finally replaced by a coarse reticulum of delicate protoplasmic strands. This network unites with the nuclear wall on one side, and joins the more peripheral and unchanged portion of the perinuclear zone on the other. The reticulum is therefore from its genesis wholly of cytoplasmic origin.

While the changes in the perinuclear zone are occurring as above described, certain developmental modifications may also be observed in the nucleus. Until this time the linin of the nucleus has not been noticeable, but now it assumes a more or less granular appearance and stains feebly, and the nuclear wall shows indication of approaching disintegration. Delicate threads of protoplasm run out from it which join, on the outside, the circumnuclear reticulum, and on the inner side, the linin meshwork. Shortly the nuclear wall becomes entirely broken down and a continuous protoplasmic reticulum occupies the entire cell.

The relation of the karyoplasmic reticulum to the perinuclear zone in the formation of the achromatic figure will be spoken of directly, and it may be premised that it is the same as is found in *Cobaea scandens* ‡ and in *Lavatera*, § as well as in other plants, that is the achromatic figure has elements drawn both from the cytoplasm and from the nucleus.

The topographic relations of the cell constituents at this time will be understood by referring to *f. 7*. When such a section is treated with the Flemming triple stain, the perinuclear zone will be colored deep orange, the reticulum within the perinuclear zone will be colored lightly with the gentian-violet, and, finally, the chromatin will of course take the saffranine.

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* Guyer. Science, II. 11 : 248. 1900.

† Strasburger, Histologische Beiträge, 6 : 42. 1900.

‡ Lawson, Some Observations on the Development of the Karyokinetic Spindle in Pollen-Mother-Cells of *Cobaea scandens*. Proc. Calif. Acad. Bot. III. 1 : 169. 1898.

§ Byxbee, *l. c.*

Prior to the breaking down of the nuclear wall the nucleolus (plasmosome) presents indications of its disintegration. Its capacity for absorbing the stains is lessened, it becomes vacuolated, and at length disappears. The degeneration of the nucleolus takes place *pari passu* with the formation of that portion of the nuclear reticulum which is of karyoplasmic origin, and it may, in fact, contribute to its building up, just as occurs according to Strasburger in *Fucus*.*

The net-work which arises from the linin of the nucleus, from the cytoplasm immediately surrounding it, from the disintegrated wall, and finally, perhaps, from the nucleolus also, becomes modified so that a confused mass of fibers are formed which make the felted condition characteristic of the earlier stages in the organization of the multipolar achromatic figure in the higher plants. The fibrillae which come from the reticulum become gathered into groups with the effect that from the felted condition a multipolar one ensues, and from this at length the bipolar figure is formed. Several intermediate stages were observed in the transformation just spoken of, such as the one figured (*f.* 8). The cytoplasmic cones, which are so prominent a feature in the early stages of such an achromatic figure as in *Equisetum* † are wholly wanting in the cotton hybrid. The presence of the perinuclear zone of cytoplasm, as has been pointed out in *Lavatera*,‡ appears to inhibit the formation of the *Equisetum* type of multipolar figure.

When the achromatic figure is completely differentiated, the poles extend nearly or quite to the periphery of the cell, and the relation between the achromatic figure and the ectoplasm is the same as described by Lloyd § as occurring in *Asperula*. In the cotton the relation is a variable one, and appears to depend on the size of the cell, as for instance, in the spindles of the second division the poles do not reach to the periphery but terminate in the outer portion of the cytoplasm. The achromatic figure of the cotton hybrid, as in pure races, is made up of three sorts of fibers, namely, of the continous fibers, or central cylinder, the contractile

* Strasburger, Kerntheilung und Befruchtung bei *Fucus*. Jahrb. Wiss. Bot. 30: 351. 1897.

† Osterhout, Ueber Entstehung der karyokinetischen Spindel bei *Equisetum*. Jahrb. Wiss. Bot. 30: 159. 1897.

‡ Byxbee, *l. c.*

§ Lloyd, *l. c.* 70.

fibers, and the mantle fibers. The continuous fibers and the contractile fibers arise from the intra-zonal network in a manner above described, but the mantle fibers appear last, they are composed wholly of cytoplasmic material, and apparently are not at all concerned with the splitting or separation of the chromosomes. The mantle fibers give rise to the fibrillar layer which was spoken of above and which is a conspicuous element of the cell after the first nuclear division.

The chromosomes become arranged so as to form a plate midway between the poles of the bipolar spindle and at right angles to the spindle. In the metaphase of the first division the chromosomes are usually ring-shaped but vary more or less as in the metaphase of this division in pure races of plants. However, when first arranged in the plate the ring form may not appear, but it does subsequently, during the process of the splitting of the chromosomes. I was not able to determine with certainty whether the first split separated chromosomes which corresponded to the two halves of the heterotype ring, or whether the split followed the second cleavage. If the former occurs, then according to the hypothesis advanced in the preceding part of this paper, the daughter nuclei are pure as regards the chromatin content. If, however, the latter is the case, the nuclei of the daughter cells would be of mixed descent. The tetrads in either case* would contain chromatin which had descended in a linear manner from either and not both of the parents.

In the anaphases of this division the chromosomes are U-shaped, V-shaped or X-shaped as in pure forms. The second longitudinal splitting which was observed in the prophases, could not be clearly made out in the anaphases because the chromosomes were so small and dense. By repeated counting of the chromosomes, I determined that there was an equal distribution of them in the first mitosis: the reduced number was 28.

In late anaphase the chromosomes take a position at some distance from the poles, so that when the reconstruction begins the ends of the spindle extend beyond (*f. 12*).

Certain characteristics of the achromatic figure may be mentioned in this place. The mantle fibers extend from the poles, at

*It is conceivable, however, that a *different orientation* of the heterotype rings might give other results.

a sharp angle to the spindle, well into the cytoplasm, and make up the fibrillar layer which is so conspicuous in the cells. Those of the fibers that are in the immediate neighborhood of the daughter nuclei soon lose the filar appearance, become structureless and the chromosomes in early telophase lie freely in an apparently homogeneous substance.

The reconstructed nuclei possess a distinct nuclear wall, the chromatin forms a coarse reticulum (*f. 12*) and the nucleolus is reconstructed. The steps in the reformation of the daughter nuclei were not closely observed but appeared roughly to correspond to the reverse of the breaking down of the mother nucleus.

When the daughter nuclei are fully reconstituted the fibers of the achromatic figure may still be seen in the inter-nuclear cytoplasm; they persist during the division of the nuclei and even may be observed in the cytoplasm of the young tetrad. The presence of these spindle-fibers in normal mitoses, and the total absence of them in abnormal ones, form a reliable basis for judging whether the mitosis was normal or abnormal, but this of course would probably not be true if the divisions were so nearly normal as some described by Juel, Guyer and Metcalf. Such, however, are not to be found in the cotton hybrid.

The daughter nuclei are so small that it is difficult to study at all closely the sequence of changes which lead up to and accompany their division, and, therefore, I contented myself with observing certain definitive phases only.

Continuous, contractile and mantle fibers are of course present in the achromatic figure, but the latter do not eventually form a fibrillar layer in the cytoplasm as the mantle fibers of the preceding division do. The continuous fibers persist and may be seen along with those from the first division in the young tetrad.

In the prophases of this division the chromatin masses arrange themselves in a nuclear plate as in the former division, but there is a distinct difference between the chromatin of the first and of the second mitosis. In the prophases of the second mitosis the chromosomes are of a uniform size and appearance and are rod-shaped. I was not able to make out the presence of the second and precocious split which in some forms may be observed at this time. In the metaphase each chromosome divides into two equal por-

tions, and these go to the opposite poles of the spindle and there build the tetrad-nucleus.

Although in many regards the description of the mitoses just given must be considered as a meager one, I think that enough has been said to show that they are the exact homologues of these nuclear divisions in pure races of plants. That is, in the cotton hybrid the first maturation is a heterotypic one and the second is homotypic.

My material was not of sufficient amount or kind to permit me to study the subsequent nuclear division of the tetrad, or the germination of the spore.

The division of the cell does not take place until the daughter nuclei are formed and completely reconstructed. When the division occurs the planes of division are at a sharp angle with one another and pass between the daughter nuclei, and the result is an equal apportionment of the cytoplasm among the four nuclei of the group. Indentations appear in the periphery of the cytoplasm and midway between the nuclei, which deepen into constrictions, and finally accomplish the separation of the nuclei and the formation of the tetrads.

A cell-wall is not organized until the tetrads are built and the formation of the wall takes place as follows: colorless accretions appear on the periphery of the tetrad; later a delicate membrane may be distinguished connecting them. Both the thickened portions and the membrane are evidently produced by secretion and respond to the test for cellulose. Somewhat later the spore-wall becomes relatively thick by the addition of the cuticularized exine; the places where the cellulose accretions first appear mark the location of the germinal pores.

Abnormal Nuclear Divisions. — The foregoing account of the tetrad formation in hybrid cotton is based on the study of anthers which were among the first to be put up, namely October 29, and were taken from the form known as "34*b*." A study of material collected in November and December showed that most of the male cells were perfectly normal. The flowers gathered in January were unfortunately older, and did not therefore show the division stages, but the appearance of the cytoplasm as well as of the nuclei was such as to indicate that normal mitoses had taken

place. In all of the material, however, which was gathered later in the season, and which had nuclei in the process of division at the time of putting up amitosis occurred. I have not been able satisfactorily to determine whether these abnormal divisions were due to the cultural conditions, to the fact that the plant was a hybrid, or to both these factors with the added one that the flowers were the last to form on the plants.

A sharp distinction was made in the cotton between the nuclei of the abnormal cells which accomplished division, and those that were abnormal but degenerate and never underwent division either by the direct or by a modification of the indirect method. Degeneration of the sex cells, either of the archesporium or in later stages, could be found to some extent in practically all of the material examined, but this was always so well marked structurally that no confusion arose by which the degeneration of the cells was questioned.

When the degeneration took place in the archesporial cells it was very noticeable both in the nucleus and the cytoplasm. The cytoplasm assumed various conditions and structures. It was a coarse reticulum of rather delicate meshes which had a uniform appearance as if the microsomes were wanting, or a large vacuole occupied the center of the cell, pushing the nucleus to one side and causing the cytoplasm to gather in various parts of the cell. The cytoplasm of the degenerate cells takes stains with great avidity. This is especially to be noticed in the anthers that have both normal and degenerate nuclei. When in such anthers the cytoplasm of the normal cells is properly stained that of the degenerate cells will always be badly overstained. This is very different from the staining capacity of cells whose nuclei divide by the direct method, since these cells stain in a perfectly normal manner.

The nuclei of degenerate archesporial cells are often smaller than those of the normal cells in the same anther, the nuclear membrane is frequently very pronounced, and the chromosomes are of the size and number of the somatic ones. I found a few cells which were preparing for the first division and which had the two-spindle condition observed by others in hybrids; but I did not notice any such nuclei in later stages, since these cells degenerate

before the divisions are completed. It is possible that the same plants grown under other conditions might have carried the divisions farther. Besides the irregularities in division as just given, others, especially in the achromatic figure, were also noted; in some cells the multipolar spindle was seen accompanied by many small nuclei.

Another form of irregularity occurred which is not so clearly degenerate but which must also be considered a factor that leads to infertility, namely the direct nuclear divisions in cells gathered the later portion of the winter. The mother-cells of the nuclei which were to undergo direct division were apparently like their counterparts in which normal mitosis would occur except as regards the structure of the cytoplasm, which retains its primitive condition; that is, a perinuclear zone is not differentiated, and a fibrillar layer is not present. The process of amitosis consists of a division of the nucleolus, followed by an equal or an unequal direct division of the remainder of the nucleus such that a nucleolus goes to each daughter nucleus.

The first amitotic nuclear division is usually equal, and the result of the division resembles that brought about by the indirect method, and also the second division may be equal and thus a tetrad group in appearance, much like that normally organized, may be formed. Usually, however, the divisions are not equal and small nuclei are associated in the same cell-complex with larger ones. The extreme is to be seen when the nucleus of the mother-cell fragments and the cytoplasm contains numerous small nuclei. Some of the forms of direct division are shown by figures *17*, *18* and *19*.

Cell-division may or may not follow the direct nuclear divisions. If it does, tetrads which are like the normal ones except as to the presence of small nodes will be formed; if, however, cell division does not take place, monstrous pollen grains result. Since the tetrads formed as a result of direct division may be like those organized in the regular manner a mere macroscopic examination of the mature pollen will give no indication of the genesis of such, and hence, it may be supposed, of its ability to germinate.

By way of general summary, I may say that in the anthers of the hybrid cotton one finds both normal and abnormal conditions.

The first maturation division in the normal cells is heterotypic, the second homotypic; and also amitosis occurs. Many additional abnormalities in the nuclei were observed; such nuclei, however, do not undergo division, but degenerate in the archesporial cell or some later stage, in every case before the first division. Among such irregularities a few mother-cell nuclei were observed with two spindles and the somatic number of chromosomes.

COLUMBIA UNIVERSITY, 1902.

Explanation of Plates

PLATE 7

FIG. 1. Microspore mother-cell.

FIG. 2. Same.

FIG. 3. Split spireme in prophase of first division.

FIG. 4. *a*, *b*, *c*, *d*, *e*; typical chromatin loops from the prophases of the first division, showing the double splitting.

FIG. 5. Same at a later stage.

FIG. 6. Condensation of rings into chromosomes preparatory for the first division; breaking down of the nuclear wall.

FIG. 7. Same, later stage.

FIG. 8. A multipolar spindle which is becoming a bipolar one.

FIG. 9. Metaphase of the first division.

FIG. 10. Anaphase of the same division, somewhat to one side of the median line; in the section next to this one the poles of the spindle were observed to extend quite to the periphery of the cell.

PLATE 8

FIG. 11. Chromosomes in the metaphase of the first division.

FIG. 12, *a*. Resting daughter-nucleus. *b*. Sketch of same nucleus, from the next section, showing its relation to the spindle, and that of the latter to the periphery of the cell.

FIG. 13. Daughter-nuclei; the reticular character of the cytoplasm not well shown; the presence of inter-nuclear fibrillae, the remains of the continuous fibers, is indicated.

FIG. 14. Early anaphase of second division; the more heavily shaded portion of the cytoplasm indicates the position of the perinuclear zone; the fibrillar layer in the cytoplasm is shown.

FIG. 15. Metaphase of the second division.

FIGS. 16–19 are of abnormal (amitotic) nuclear division of spore mother-cells.

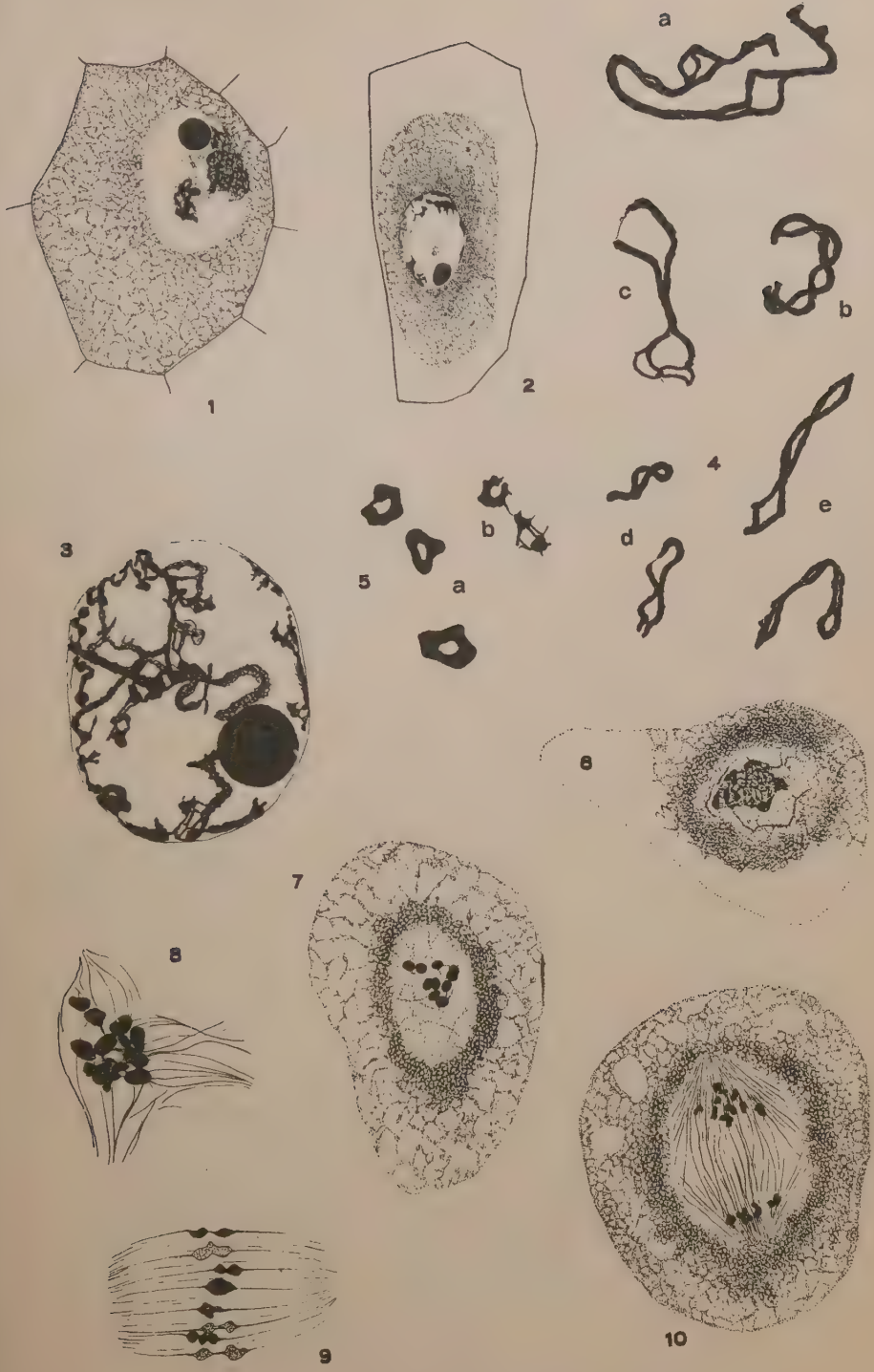
FIG. 16. Daughter-nuclei.

FIG. 17. Nuclei of different sizes.

FIG. 18, *a* and *b*. Tetrads with smaller nuclei scattered in the cytoplasm.

FIG. 19, *a* and *b*. Equal and unequal direct division of the nucleus of the mother-cell.

NOTE. — The primitive character of the cytoplasm is shown in Figs. 16, 17, 18*a* and 18*b*.



SPERMATOGENESIS OF HYBRID COTTON.

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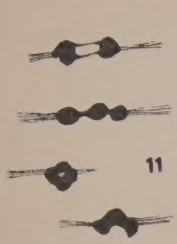

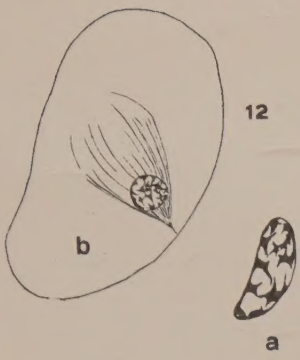

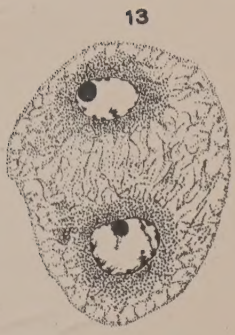

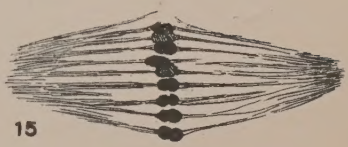

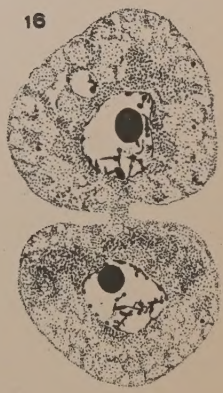

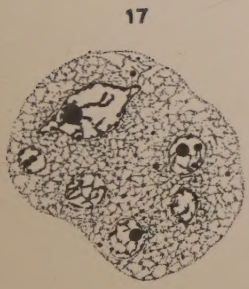

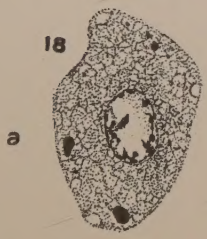

SPERMATOGENESIS OF HYBRID COTTON.

HELIOTYPE CO., BOSTON.